AN X CHROMATIN SURVEY AT WOODWARD STATE HOSPITAL AND TRAINING SCHOOL, AN INSTITUTION FOR THE MENTALLY RETARDED

An abstract of a Thesis by
Janet Kay Lyman
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Drake University
Advisor: Michael E. Myszewski

The problem. The majority of the Woodward State hospital and training school population was surveyed to locate and identify residents with X chromatin abnormalities.

Procedure. Buccal smears were taken from each resident. The smears were stained with cresyl fast violet and examined under 1250X magnification. The number of Barr bodies per 100 cells was noted and recorded for each resident sampled.

Findings. One male out of a total male population of 278 surveyed was found to be chromatin positive, a frequency of 0.4%. Since four cell lines were observed in the buccal smear analysis, he was classified as a mosaic for his X chromosome makeup. Analysis of a population of 193 females showed one female with an abnormal X chromatin count, a 0.5% frequency. Since this female's buccal smear count was just slightly lower than that considered normal, she was classified as a mosaic of the type 45,X/46,XX.

Conclusions. The frequency of X chromatin males at WSHTS was generally lower than the frequencies found in surveys of residents in other institutions for the mentally retarded. The frequency of females with abnormal X chromatin counts at WSHTS was in close agreement with that found in other similar surveys.

Recommendations. The residents found in the WSHTS survey with abnormal X chromatin counts should be investigated by karyotype analysis to determine the specific features of the abnormality.

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Janet Kay Lyman

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Janet Kay Lyman

Approved by Committee:

Dr. M. E. Myszewski
Chairman

Dr. H. D. Swanson

Dr. Joe Woods

Earle L. Canfield
Dean of the School of Graduate Studies

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INTRODUCTION AND REVIEW OF THE LITERATURE

Barr and Bertram (1949) discovered the X chromatin body or Barr body during their observations of the hypoglossal nuclei of the female cat. In their examination of sympathetic ganglia of human males and females they reported a similar sex difference in the human ganglion cells. Subsequent observations of cells obtained from autopsies confirmed an X chromatin mass in cells of the thyroid gland, cartilage, the adrenal cortex, smooth muscle, and the bladder (Barr and Moore, 1954).

Barr and Moore (1955) reported an X chromatin difference in male and female epidermal cells. Davidson and Smith (1954) demonstrated a sex difference in human polymorphonuclear leucocytes of peripheral blood. The female granulocytes displayed an appendage attached to the body of the nucleus: this appendage is now called a drumstick or sex nodule.

In the cytogenetic laboratory, the presence or absence of the X chromatin may give the first information regarding the numbers of X chromosomes present in patients suspected of carrying X chromosome abnormalities (Mittwoch, 1967a). Because of its accuracy and simplicity, the buccal smear technique for detecting X chromatin bodies is an effective test when used in the general screening of selected or random populations to uncover X chromosomal abnormalities

(Pansegrau and Peterson, 1964).

The buccal smear technique was used in the present survey to screen residents at Woodward State Hospital and Training School (WSHTS). The test results will eventually serve as a guide for the diagnosis and management of X chromosomal disorders.

Quite frequently the term sex chromatin is used to signify X chromatin or Barr body. The terms X chromatin body or Barr body may be used synonymously; however, sex chromatin is a misleading term since it may refer to either of the two unlike sex chromosomes in man i.e., Y body or X chromatin body which differ in several important respects (Polani, 1969). The Y body is not present in female cells and until recently was difficult to detect in male cells. There has lately been developed a fluorescent staining technique by which cases of multiple Y chromosome abnormalities can be detected (Cervenka, 1971).

Grumbach et al. (1962) found the X chromatin body to be a late DNA replicating chromosome in the 6-12 group. They applied tritiated thymidine to cultures of human leucocytes before the end of the period when DNA is synthesized and found most of the chromosomes to have completed their DNA synthesis. This technique revealed that in cells from normal females one chromosome is heavily labeled. Since the heavily labeled chromosome is not found in males it was assumed to be the second X chromosome.

This conclusion was further strengthened by Comings (1967) who demonstrated that the inactive X chromosome does not begin DNA replication until 2.5 hours after the onset of euchromatin replication. The term euchromatin refers to those chromosomal regions that make up the fine network of chromatin in the interphase nucleus and, in contrast to the inactive X chromosome, does not stain darkly nor remain condensed (Levitan and Montagu, 1971).

Moore (1966) believed DNA synthesis to be difficult for the X chromatin body in the condensed state. He hypothesized that the short segment of the X alternately despiraled, replicated, and condensed again. However observations by Klinger et al. (1967) indicate the condensed sex chromatin forming the X chromosome does not despiral completely during DNA replication.

The number of late-labeling X chromosomes is the same as the number of Barr bodies, one less than the total number of X chromosomes in the nucleus. These late-labeling chromosomes are believed to form the Barr bodies or X chromatin bodies (Mittwoch, 1967b).

The late-labeling X chromosomes appear as darkly condensed masses of chromatin material when stained. These masses were counted and recorded in the WSHTS buccal smear survey.

The Barr body is a small planoconvex body lying against the inner surface of the nuclear membrane of the

nucleus of normal females. It is present in a large proportion of nuclei of female origin but absent in normal male nuclei (Carel and Levij, 1968).

The size of the Barr body is estimated as 0.7 X 1.4 microns in diameter both in nuclei of the buccal mucosa and in sections of several human tissues. Interphase nuclei of either sex may contain darkly staining bodies known as chromocenters; however, they are smaller than Barr bodies and are less well defined. These chromocenters may mistakenly be interpreted as Barr bodies in male nuclei (Carel and Levij, 1968).

Based on thy evidence that one X chromosome of the female forms the X chromatin and from the patterns of mosaicism observed in the expression of X linked loci in the mouse, Lyon (1962) proposed that the cytological manifestations coincide with a genetic inactivation of either the maternally or the paternally derived X chromosome in an early stage of embryological development. Once the change is induced, the descendants of each cell maintain that state.

Mittwoch (1964) measured the size of nuclei in human fibroblast cells undergoing DNA synthesis. She found in all DNA classes the nuclei with multiple chromocenters were smallest, the cells with a single Barr body were intermediate in size, and those lacking a Barr body were the largest. She suggested the smaller nuclear size is partly a direct

effect of the condensation of one X chromosome and partly an indirect effect. There was no explanation regarding the possible indirect effects that could influence nuclear size.

The frequency of the Barr bodies observed in cells of the oral mucosa varies greatly. Moore (1966) estimated the percentage of Barr bodies in normal XX chromosome complement females to be between 20-70%; however, values above 60% are rare. Beaver and Douglass (1969) examined 257 buccal smears and estimated the percentage of positive cells in normal females to be 2-23%. Their lower X chromatin values resulted from the inflexible use of more rigid criteria than previously used by others. Pansegrau and Peterson (1964) reported an average X chromatin frequency of 97.6% in female buccal cells. They, unlike the previous studies mentioned above, included both peripheral and central chromatin bodies in their counts; this would explain the elevated frequency of Barr bodies.

Technical factors may influence the number of nuclei found to show an X chromatin body. In the cresyl fast violet technique the deep staining of the mouth bacteria may mask the nuclei in human buccal smears. Overlapping cells distorting the nuclei and folded nuclei contribute to the number of unsuitable cells that can not be counted. If the microscope slides containing the buccal smears are allowed to dry and not fixed immediately the cells and their nuclei are torn and impossible to analyze (Mittwoch, 1967a).

The incidence of X chromatin varies under different conditions in the same tissue. Variations were found by Blanco de del Campo et al. (1965) in X chromatin counts in buccal smears during the menstrual cycle; however, this was not confirmed by Brainerd et al. (1965). Lower X chromatin counts were noted during treatment of females with sex hormones. The lower X chromatin values during hormonal treatment may be the result of changes in nuclear size which reportedly occur under the influence of hormones (Carel and Levij, 1968).

In the normal human female only one X chromosome is visible in the buccal smear; however, two X chromosomes are present in the cell nucleus. This can be attributed to the inactivation and ultimate condensation of one of the X chromosomes in each cell. To determine the number of X chromosomes in each cell the N - 1 rule applies, where N equals the number of X chromosomes and N - 1 equals the number of Barr bodies in the buccal smear (Pansegrau and Peterson, 1964).

In the male the same rule applies. The normal male (XY) should show no Barr bodies; however, there are males that exhibit an X chromatin mass. These males may have two or more X chromosomes accompanied by a Y chromosome in their body cells. To determine the number of X chromosomes present in the male the N - 1 rule is used i.e., an XXY male presents one Barr body (Pansegrau and Peterson, 1964).

A Turner syndrome female demonstrates no Barr bodies and has an XO or 45,X chromosome complement. These persons are phenotypically female, but they do not develop the secondary sexual characteristics common in normal females. They may have broad shoulders, small and lateral nipples, thorax developed like a shield, and they will be sterile. Other physical characteristics may include short stature, webbing of the neck, coarctation of pulmonary arteries, and, rarely, mental retardation. The frequency of XO types in the general population is one per 3000 (Levitan and Montagu, 1971).

The triplo X chromosome complement (47,XXX) is observed as double Barr bodies in buccal smears. These individuals are phenotypically female and may appear to be normal. Physical abnormalities that may accompany this disorder are obesity and a tendency toward webbing of the neck. The secondary sexual characteristics may be poorly developed. The frequency of XXX types is 1.2 per 1000 live births (Barr et al., 1969).

A variation of the standard X chromosome abnormalities is the 46,XX/47,XXX mosaics. Individuals with this abnormality show a variety of physical symptoms depending on the site and size of the XX clone. The buccal cells may show a high percentage of cells with one X chromatin body and a lower percentage with two Barr bodies. A representative example of an XX/XXX mosaic was found in a population of

4514 mentally retarded residents in 15 independent institutions (Baikie et al., 1962). Sixty five per cent of this patient's buccal cells showed a single X chromatin body and 23% showed two bodies. Analysis of skin cells revealed one cell line only: 47 chromosomes and an XXX complement. There was evidence for two cell lines in a blood culture: an XXX line and an XX line (Baikie et al., 1962). More than one cell line should be examined in suspected mosaics because the results of studying the chromosomes of one cell type may not be duplicated in another cell line (Barr et al., 1969).

In Klinefelter syndrome males, a chromatin positive single Barr body is present, reflecting an XXY chromosome complement. The physical characteristics of these males include scanty body hair, female breast development, small aspermic testes, and sterility. This syndrome is often accompanied by mild mental retardation. The frequency of XXY types in the general population is 2.1 per 1000 (Cotter et al., 1960).

Males may also show more than one Barr body per cell when more than two X chromosomes are present (Baikie et al., 1962). Each additional X chromosome contributes another Barr body, so that XXXY males show two; and XXXXY males show three (Levitan and Montagu, 1971). These males will show a significant number of Barr bodies in their buccal mucosal cells if the extra X chromatin bodies are present in the chromosomes of the mucosal cells (Baikie et al., 1962).

There is little precise information about the effect on intelligence of carrying more than three X chromosomes but, the tendency seems to be that the more X chromosomes there are, the greater is the depression of intellectual level (Polani, 1969). The newborn incidence of XXXY males is very low; no case was detected in more than 18,000 male births (Polani, 1969).

The XY/XXY male mosaic will have a number of cells with one X chromatin body present (Polani, 1969). Individuals of this karyotype differ from the typical XXY Klinefelter in a number of important respects. Small testes are present in only about 80% as compared with 100 per cent in XXY's; aspermia occurs in about 60 per cent instead of 90 per cent; and 30 per cent are presumptive fathers as compared with only three per cent in the other group. Sub-normal intelligence is significantly less frequent (Levitan and Montagu, 1971). The newborn incidence of XY/XXY mosaicism is 0.07%. This type of abnormality may be between five and six times more common in hospitals for the mentally retarded than among newborn males, possibly due to the admixture of cells within the brain contributing to the inability of these patients to adapt socially (Polani, 1969).

Mosaics of the form XXXY/XXXXY should have a higher proportion of cells with two bodies present and a lower proportion of cells with three bodies present. A buccal smear survey by Baikie et al. (1962) revealed 44% of the

buccal cells contained one body, 41.5% contained two bodies, and three bodies were found in 11.5% of the buccal cells of this male mosaic. A chromosome analysis of the blood leucocytes showed 69% of the cells to be consistent with an XXXY complement and 28% of the cells to be consistent with an XXXXY complement. None of the blood leucocytes showed an XXY complement. In mosaics the admixture of the component cell lines may vary widely from tissue to tissue; therefore a group of subjects with the same type of mosaicism may show considerable phenotypic variations, and a proportion may have a phenotype indistinguishable from normal (Polani, 1969).

An extensive X chromatin survey with follow up karyotype analysis was conducted on 15 institutions for the mentally retarded with a total of 4514 residents (Baikie et al., 1962). The results are as follows: 28 out of 2607 males were chromatin positive or a frequency of 1.07%. Twenty-three of these males had one X chromatin body in a majority of their cells, four had some cells containing two bodies, and one had three bodies in a proportion of buccal cells.

Among 1907 females in the same survey, nine had abnormal X chromatin counts, a frequency of 0.5%. Eight of these females had two X chromatin bodies in a proportion of their buccal cells, while only one female had no X chromatin bodies in the majority of her buccal cells (Baikie et al., 1962).

Hamerton et al. (1962) studied a population of 425 mentally defective children: 229 males and 196 females. They found by karyotype analysis one male, an XXY, and one female, an XXX, with an X chromosome abnormality. Another female was found to have several somatic characteristics of Turner syndrome but with a normal female X chromatin pattern. The frequency of abnormal X chromatin counts in this female population was 0.5%: the frequency of chromatin positive males was 0.4%.

In an X chromatin survey conducted at a state institution in Iowa quite similar to WSHTS, 733 patients were tested by the buccal smear method (A Sex Chromatin Study at an Institution for the Mentally Retarded, Eisen and Stinard, unpublished, 1971). Five males out of 452, 1.11% frequency, had abnormal X chromatin counts. Four of these males were mosaics: the fifth displayed Klinefelter syndrome.

Of the 281 females tested by Eisen and Stinard (unpublished, 1971) ten were found to have abnormal X chromatin counts, a 3.5% frequency. Nine of these females were mosaics: five were 45,X/46,XX mosaics and four were 46,XX/47,XXX mosaics. One female was found to be a possible XO Turner syndrome. The criteria used by Eisen and Stinard for the recognition of the X chromatin body was not stated, nor were the X chromatin counts of the abnormal patients given in their report.

Cotter et al. (1960) surveyed 1252 male patients out of a popluation of 1500 mentally retarded males. Determination of the X chromatin pattern was done by the buccal smear technique. In those patients found to have a positive X chromatin pattern further clinical and laboratory studies were done including testicular biopsies. Ten of the male patients examined were positive for the X chromatin, XXY, with a frequency of 0.8%.

Boyd et al. (1960) studied 595 buccal smear preparations out of a female population of 637 mentally retarded patients. Four patients displayed an abnormal X chromatin pattern which was later substantiated by karyotype analysis. All four females were 47,XXX, a frequency of 0.7%. They found no consistent physical symptoms in those females displaying the XXX syndrome.

An extensive survey was conducted by Lubs and Ruddle (1970) on 4366 infants born consecutively over a period of one year. Twenty-two infants were born with a chromosome abnormality. Eleven of these involved the sex chromosomes, a frequency of 0.25%.

Of the 218 male infants surveyed by Lubs and Ruddle (1970), the XYY karyotype was found in three with a frequency of 0.14%. Four X chromatin positive males were found in this newborn population, a frequency of 0.18%.

Polani (1969) pooled the results of studies on newborn infants from Canada, England, Scotland, Switzerland,

and the United States giving a total of more than 40,000 infants of both sexes. He calculated one in 465 or 0.22% live born males to be chromatin positive: two thirds of these 0.22% males or 0.15% were XXY and one third of the 0.22% or 0.07% were mosaics.

The number of newborn females found by Lubs and Ruddle (1970) to have abnormal X chromatin counts was four out of 2182 or 0.18%. An XXX karyotype occurred once in 727 female newborns, and a 45,X karyotype only once in 2182 female newborns.

Polani (1969) calculated the frequency of newborn females with an abnormal X chromatin count to be 0.12% in his pooled data. He found, in newborn females, one in 2574 to be 45,X and one in 1253 to be 47,XXX. Polani's (1969) estimate is lower than but in general agreement with the results of the Lubs and Ruddle (1970) survey.

The proportion of XXY males in hospitals for the mentally retarded is four times the proportion of XXY males at birth, based on data from 17,000 males (Polani, 1969). Since the degree of intellectual impairment in chromatin positive males with a single X chromatin mass tends to be relatively mild, their presence in institutions may be determined by their social behavior and not on their chromosomal abnormality (Polani, 1969).

Male mosaics may be between five and six times more common in hospitals for the mentally retarded than among

newborn males (Polani, 1969). While chromosome mosaics seem to have a higher intelligence quotient than chromosomally abnormal non-mosaic subjects, it could possibly be that the admixture of cells within the brain has an adverse effect on its more subtle functions and on the social adaptations of mosaic individuals; which might explain their relative frequency in hospitals for the mentally retarded (Polani, 1969).

The overall prevalence of 47,XXX females in hospitals for the mentally retarded is five times their incidence at birth (Polani, 1969). The triplo X chromosome error predisposes the individual to mental retardation and mental illness, hence their higher incidence in hospitals for the mentally retarded than the newborn frequency would indicate (Barr et al., 1969).

The frequency of 45,X females in institutions for the mentally retarded is 0.02% which is lower than their incidence at birth (0.04%) (Polani, 1969). This would indicate that women with an XO chromosome complement are not appreciably mentally handicapped, therefore there is no tendency for XO women to be admitted to hospitals for the mentally retarded (Baikie et al., 1962).

Females with X chromosome mosaicism involving a 45,X cell line have been found with a frequency of one in 1402 or 0.07% in hospitals for the mentally retarded (Polani, 1969). There is no reliable data on the prevalence of these

mosaics in the newborn population because in infants who are XO/XX mosaics, X chromatin bodies are usually present; therefore, these infants will not generally be detected as a result of X chromatin screening of large populations of newborns (Mittwoch, 1967a). It is generally supposed, however, that the incidence in institutions is slightly higher than in the newborn population. Female mosaicism of this type may parallel male mosaicism in that the several cell lines within the brain may have an adverse effect on the social adaptations of these individuals (Polani, 1969).

X chromatin surveys have previously been conducted on selected individuals at Woodward State Hospital and Training School (WSHTS); however, the present survey will be more comprehensive and will update the available information by including a majority of the residents.

A total X chromatin picture of the institution would be valuable in correlating the genetic configuration of individuals with physical and mental abnormalities.

In the past there have been no Drake University students involved in genetic studies of the residents of WSHTS; therefore, this will be the first in a series of projects conducted by graduate students in genetics. This survey will identify individuals with abnormal X chromatin counts who will be karyotyped in a follow up study. The combined information resulting from the total study conducted at WSHTS could lead to a greater insight into,

and increased knowledge of, human genetic abnormalities.

MATERIALS AND METHODS

The population of Woodward State Hospital and Training School (WSHTS) in Woodward, Iowa is approximately 750-800 residents and 471 of these were involved in the present sampling. Approximately two thirds of the parents contacted through letters distributed by the WSHTS Administration Office granted permission for the survey to be conducted.

Sampling of the residents at WSHTS was done each Friday beginning in January 1972 and terminating in April 1972. Sampling consisted of taking two buccal smears from each resident and placing each smear on a separate microscope slide. One or both of the slides were examined for the presence or absence of Barr bodies.

The mucosa of the cheek was scraped firmly with the edge of a wooden tongue depressor. The material obtained was smeared gently on microscope slides. The cells were fixed immediately in a mixture of equal volumes of absolute ethanol and ether for at least 15 minutes. The fixed smears were passed through 70% alcohol and 50% alcohol and two changes of distilled water (Barr and Moore, 1955). The cells were stained in cresyl fast violet solution six minutes and differentiated by dipping the slides quickly five to seven times in 95% alcohol. The slides were dehydrated

in absolute alcohol for one minute and mounted in Euperol (Moore, 1966).

The smears were examined with oil immersion optics using a Zeiss Photomicroscope II. Bright field optics were used and a magnification of 1250X was employed for counting the cells.

Several criteria were used for the recognition of the Barr body. In order to be considered suitable for examination the Barr body had to be free of disconcerting artifacts such as precipitated stain, other foreign material, and bacteria. Cells unsuitable for counting were those exhibiting folding of the nuclear membrane or overlapping cells that masked the Barr body. The cell was considered positive for the X chromatin body only if the nucleus contained a condensation of chromatin material that was planoconvex to triangular in shape and closely applied to the nuclear membrane.

The result of the buccal smear examination for each resident screened was recorded in one of the three following ways:

1. Positive for the X chromatin body. This diagnosis was used when more than 1% of 100 cells on a slide fulfilled the criteria described previously.

2. Negative for the X chromatin body. This diagnosis was used if less than 1% of 100 cells displayed an X chromatin mass.

3. Insufficient material. This diagnosis was used if no X chromatin bodies were seen, but less than 100 suitable prepared cells were present.

RESULTS

Sixty-three per cent (471) of the January 1972 WSHTS population (750) were examined in this X chromatin survey. Of the 471 residents sampled, two hundred seventy eight (59%) were males, representing 57% of the total male population at WSHTS. One hundred ninety three (41%) of the residents sampled were females or 54% of the total female population.

The average number of Barr bodies per cell in the female population was 26.01 $\pm$ 5.73. All counts under 14.55, two standard deviations below the mean average, were considered abnormal.

Male X chromatin counts were considered questionable and in need of further investigation if more than one X chromatin body was observed in 100 cells.

TABLE 1. DISTRIBUTIONS BY SEX OF RESIDENTS WITH NORMAL AND ABNORMAL X CHROMATIN COUNTS

Sex	Normal	Abnormal	Total
Male	277	1	278
Female	192	1	193
Total	469	2	471

One male out of 278 was found to be chromatin positive. Forty-nine out of 100 buccal cells of this individual were found to contain one X chromatin body, 28 cells out of 100 had two X chromatin bodies, and one cell out of 100 exhibited three X chromatin bodies. The clinical diagnosis of this individual was encephalopathy due to asphyxia at birth.

With one of 278 males demonstrating a deviation, the overall frequency of X chromatin positive males in the residents studied was 0.4%. Furthermore because of the several cell lines observed in this individual he may also be classified as a mosiac for his X chromosome complement.

One female out of 193 was found to have an abnormal X chromatin count. This patient was a possible mosaic of the form 45,X/46,XX based on the X chromatin frequency of 14 per 100 cells. The clinical diagnosis for this individual was encephalopathy due to asphyxia at birth. The overall frequency of females with abnormal X chromatin counts was 0.5%.

TABLE 2. SUMMARY OF CASES FOUND TO BE ABNORMAL

Case Number	Birth Date	Sex	AAMD Classification	Diagnosis based on X chromatin
1	2-28-54	F	Encephalopathy	Mosaic
2	11-14-53	M	Encephalopathy due to asphyxia	Mosaic

DISCUSSION

Four hundred seventy one of the 750 residents at WSHTS were included in this survey. The total number of residents is an approximate figure due to the fluctuation of patients into and out of the institution. This fluctuation was responsible in part for the inability to obtain samples from the entire WSHTS population. The survey was also limited to only those residents having permission slips from parents or guardians for the testing to be done. If at all possible, all residents with permission slips were sampled; however, those patients that were on vacation, permanently released from the hospital, on an extended work release program, or deceased during the course of the study were not involved in the sampling. Unavailable patients amounted to a very small per cent of the total population with permission slips.

The cresyl fast violet technique for staining the buccal cells was chosen because it was most suited to the time elements involved in obtaining the smears. The entire staining process takes a minimum of nine minutes excluding fixation time of at least 15 minutes. This fixation time coincided with the amount of time needed in each ward for sampling of the residents. Because the staining of the slides was rapid and efficient, they could be prepared at WSHTS. The X chromatin body was easily recognized as a

dark purple body in the buccal smears. A disadvantage of this technique was the dark staining of the mouth bacteria which sometimes masked the Barr body and made analysis of some cells impossible. In order to obtain 100 suitable cells to count, two smears were taken and stained for each resident.

The frequency of X chromatin positive males in this survey was 0.4% based on a male population of 278. This frequency is lower than that obtained by Baikie et al. (1962) of 1.07% and that of Cotter et al. (1960) of 0.8%. Comparisons of population sizes in each survey may account for this discrepancy. The male population in the Baikie et al. survey was 2607 and that of the Cotter et al. survey was 1252 patients. Furthermore the Baikie et al. survey obtained their results by averaging information on X chromatin positive males from 15 different institutions. The largest institutional population in their survey, 639 males, had a frequency of 1.25%; whereas, the smallest male population they surveyed (49 males) had no chromatin positive males. The number of X chromatin positive males appears to decrease as the number of males surveyed decreases; therefore the variations in frequencies of the three surveys may be due to the relatively small population size of the WSHTS survey.

The Baikie et al. (1962) survey and the Cotter et al. (1960) survey were comprehensive, including all males in each

institution which increases the possibility for unbiased results. As stated previously, the present survey included 57% of the total male population.

The results of the Hamerton et al. (1962) survey (0.43% frequency of X chromatin positive males) is in close agreement with the 0.4% frequency in this study. The male populations in each study were similar in size: Hamerton et al. male population being 229, WSHTS male population being 278.

The study by Eisen and Stinard (unpublished, 1971) included a comprehensive survey of 452 males with a 1.11% frequency of males with abnormal X chromatin counts. The low number tested in the WSHTS survey may explain the differences in frequency; however, it should be noted that unlike the other studies previously mentioned the results of the Eisen and Stinard survey were not confirmed by karyotype analysis. Their frequency may therefore be greater than the actual frequency of X chromatin positive males in the institution. They also did not state as to whether rigid scoring criteria was adopted in the buccal smear analysis.

The frequency of chromatin positive males at birth is 0.19%. This is derived by averaging the data from the Lubs and Ruddle (1970) survey of 0.18% and the Polani (1969) survey of 0.22% on newborn infant boys. A comparison of the pooled data from the two surveys above (0.19%) with that of the WSHTS survey (0.4%) would indicate the incidence of X

chromatin positive males in institutions is greater than their incidence at birth. This was substantiated by Polani; however, he estimated a four to five fold increase in the number of X chromatin positive males in institutions for the mentally retarded as opposed to X chromatin positive newborn males. The per cent of X chromatin positive males at WSHTS is less than might be expected, possibly due to the low number of males surveyed.

The buccal smear analysis of the X chromatin positive male in the WSHTS survey indicated the following cell lines: 49,XXXXY/48,XXXY/47,XXY/46,XY with the majority of buccal cells exhibiting one X chromatin body. Further investigation by karyotype analysis of leucocyte chromosomes confirmed these several cell lines; however, the majority of abnormal cells contained 49 chromosomes (Dawson, 1974). The discrepancy between the buccal smear analysis of X chromatin bodies and that of the karyotype analysis of leucocyte chromosomes might be expected since in mosaics the admixture of the component cell lines may vary widely from tissue to tissue (Polani, 1969).

Dawson (1974) described the physical characteristics of the mosaic male as follows: "He was profoundly retarded with an I Q of less than 25. There was no locomotion until the age of seven months. He had an undescended left testicle and several bony defects. He had impaired motor and visual skills and did not speak. He was prone to much

self abuse as evidenced by several facial scrapes and one apparently self-inflicted cauliflower ear."

This individual's phenotypic characteristics show an extreme variation from the classic Klinefelter syndrome possibly due to the increased number of X chromosomes. The additional chromosomes would have a negative effect on mental ability resulting in this individual's lack of motor and visual skills. The variations seen in physical abnormalities might also be expected due to the several cell lines found within this male.

The frequency of mosaic males at WSHTS was determined to be 0.4%. Polani (1969) estimated a five to six fold increase in institutionalized mosaic males over newborn mosaic males. The frequency of newborn mosaic males in institutions for the mentally retarded would be calculated as a 0.42% frequency. This is in agreement with the per cent of mosaic males at WSHTS.

Mosaics exhibiting one normal cell line in general seem to be intellectually superior to chromosomally abnormal non-mosaic individuals.

In a study reported by Polani (1969), scores on intelligence tests from two groups of individuals were contrasted. One group, totaling 25, exhibited the classic Turner syndrome 45,X chromosomal count. The other group totaled 11 and were 45,X/46,XX mosaics. The mosaics had a higher mean score than the 45,X individuals. In both

groups there was a significantly better verbal than performance score. Polani indicated that it was difficult to determine what patient-selection bias might be operating and admitted it was difficult to find an easy biological explanation of the results. It could possibly be that in mosaics with one normal cell line the genetic imbalance caused by the addition of an abnormal cell line may make them less mentally acute and have a profound affect on the individual's insight, perception, and analytic ability; hence their placement in institutions for the mentally retarded.

For Klinefelter syndrome and Klinefelter variants there does not seem to be equivalent information. Gardner and Neu (1969) state that, for the Klinefelter variants, as the number of cell lines increases, because of more X chromosomes, the greater is the depression of intellectual level and the greater likelihood of their placement in institutions such as WSHTS.

An X chromatin count below 14.55, two standard deviations below the average X chromatin count (26.01) for the entire WSHTS female population surveyed, was considered abnormal. Moore (1966) estimated the per cent of Barr bodies in normal XX chromosome complement females to be between 20-70%. The average X chromatin count (26%) of females tested at WSHTS falls within this range.

Beaver and Douglass (1969) estimated the percentage of positive cells in normal females to be 2-23%. This range

would not include the 26% of the present survey; however, the more rigid criteria used by Beaver and Douglass may account for this difference. To be acceptable to Beaver and Douglass each X chromatin body had to be 1.4 microns in greatest diameter calibrated by an optical stage micrometer. Since the Barr bodies in the present survey were not measured, it is possible that some of them would not have been acceptable to Beaver and Douglass.

In contrast to the percentages mentioned above, Pansegrau and Peterson (1964) reported an average X chromatin frequency of 97.6% in female buccal cells. They used a modification of Guard's Technique in staining the buccal smears. This method involves staining of smears first in biebrich scarlet and then differentiating and counter-staining them in fast green. They reported excellent results with this staining procedure; however, Moore (1966) found the technique too unreliable to be used routinely. The criteria they used for the recognition of the X chromatin body was more flexible, including both peripheral and central chromatin bodies, than that of the present survey. This criteria and their staining procedure may explain their elevated frequency of X chromatin bodies in the buccal cells.

One female out of a population of 193 females at WSHTS was found to have an X chromatin count of 14/100, slightly lower than the criteria for normal (14.55/100). This patient appeared to be a mosaic of the form 45,X/46,XX;

however, further investigation including a karyotype analysis is recommended to determine the accuracy of the buccal smear test. Dawson (1974) in his follow up karyotype analysis of X chromatin abnormalities at WSHTS inadvertently excluded this female in his studies.

Since the X chromatin count of the female at WSHTS was not substantially lower than the criteria for normalcy, the frequency of females with an abnormal X chromatin count at WSHTS could be considered the same as the frequency of mosaics with a 45,X cell line (0.5%). This is in agreement with the Baikie et al. (1962) survey of 0.5% frequency of abnormal female X chromatin counts and the Hamerton et al. (1962) survey of 0.5% frequency.

The Baikie et al. survey included the total female population from 15 institutions or 1907 females in contrast to the Hamerton et al. survey that consisted of only 196 females, an amount similar to the WSHTS female population surveyed. It should be noted, however, that the abnormal females found in both the Baikie et al. survey and the Hamerton et al. survey were 47,XXX.

There were no female mosaics involving a 45,X cell line in either survey; however, one chromatin negative female was found in the Baikie et al. survey. There was no information given concerning the per cent of X chromatin negative cells in the patient's buccal smear analysis nor was the scoring criteria mentioned for establishing the existence

of chromatin negative females.

Eisen and Stinard (unpublished, 1971) estimated the abnormal female X chromatin count to be 3.5% out of a female population of 281 surveyed. This is a seven fold increase over the 0.5% frequency in the WSHTS survey. Eisen and Stinard explained this increased rate of abnormal female X chromatin counts as due to the low number tested and a possible drug induced artifact lowering the X chromatin counts of the females. They found five possible mosaics involving a 45,X cell line; however, these were not karyotyped and the criteria for determining mosaicism was not given in their report.

Boyd et al. (1960) found the frequency of abnormal female X chromatin counts to be 0.7% out of a female population of 595. This is in close agreement with the per cent in the present survey. Their research, however, was biased toward the 47,XXX syndrome; therefore, if 45,X mosaicism had been found it would not have been reported in their study.

Polani (1969) calculated the frequency of females with X chromosome mosaicism involving a 45,X cell line to be 0.07% in hospitals for the mentally retarded. The frequency of abnormal female X chromatin counts involving 45,X mosaicism in the WSHTS survey was 0.5%. The increase over the expected per cent might possibly be due to the low number tested in the WSHTS survey. Since this female's X chromatin count was not substantially lower than the calculated

criteria for normalcy (14.55) the patient may prove, under further investigation, to be normal. It might also be possible that in the 46% of the female population not surveyed more females with abnormal X chromatin counts might be identified.

The frequency of newborn females with abnormal X chromatin counts is 0.15%. This is an average of the 0.18% frequency calculated by Lubs and Ruddle (1970) and Polani's (1969) frequency of 0.12%, based on a combined total of 42,184 infants. Comparing this average with that of the WSHTS survey of 0.5% would indicate a substantial increase in institutionalized females with abnormal X chromatin counts over the newborn rate. This might be expected since a chromosome imbalance predisposes the individual to mental abnormality; however, the magnitude of the risk is virtually impossible to estimate (Barr et al., 1969).

Since there is no reliable data on the frequency of females with X chromosome mosaicism and a 45,X line in newborn population, no comparisons with the 0.5% frequency in WSHTS are possible. It might be assumed, however, that the frequency is higher in institutions than in newborn female infants. These mosaics may not be as mentally impaired as chromosomally abnormal non-mosaic females; however, the several cell lines within the brain may predispose these individuals to abnormal behavior and may affect their social adaptations (Polani, 1969).

The present survey will update available information

at WSHTS by providing an X chromatin screening of a majority of the residents. Through this screening process possible residents with abnormal X chromatin counts may be identified and investigated more thoroughly in future research projects at WSHTS.

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